GENETIC POPULATION STRUCTURE OF BIGHORN SHEEP AND MOUNTAIN LIONS ON THE DESERT NATIONAL WILDLIFE REFUGE, NEVADA

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EXECUTIVE SUMMARY

This report summarizes findings concerning genetic diversity and gene flow of desert bighorn sheep in the Sheep Range of the Desert National Wildlife Refuge relative to bighorn sheep in nearby ranges. A secondary topic concerned genetic diversity and relationships among mountain lions within the Sheep Range. These topics comprised components of an overarching project titled, Assessment of Desert Bighorn Sheep on the Desert National Wildlife Refuge: Phase 1.

We developed and analyzed data for 18 nuclear microsatellite loci from 185 individual bighorn sheep sampled from five mountain ranges: Sheep, Desert, East Pahranagat, Pintwater and Spotted. We then sequenced 150 of these individual bighorn sheep for mitochondrial DNA (mtDNA) control region. To determine genetic diversity and parentage of mountain lions within the Sheep Range, we developed 19 microsatellite loci and collected data from five lions found within the range and compared these to data generated for mountain lions from a more diverse population. Our assessment indicated that the mountain lions captured in the Sheep Range during this study represented three generations of one matriline. Genetic diversity was particularly low when compared to a more interconnected lion population within the Owens Valley on the eastern edge of the Sierra Nevada.

The Sheep Range has been the recipient of past augmentations of bighorn sheep. We incorporated previously generated genetic data from the major source populations to assess the influences of these augmentations on current genetic population structure. The genetic population structure of bighorn sheep in the Spotted Range appears to have predominately resulted from translocations of sheep into that range. In contrast, we observed a lack of genetic evidence from the translocations

of sheep into the southern Sheep Range. One possible explanation for this lack of genetic signature is that the population in the Sheep Range at the time was sufficiently high to greatly dilute the effect of such releases. Other possible explanations essentially suggest that the translocation efforts to the Sheep Range were failures because of potential mortalities of translocation sheep or their descendants.

We found high levels of gene flow among bighorn sheep in the Sheep, Desert, and East Pahranagat ranges based on microsatellite data. We observed unexpectedly low estimates for gene flow between those ranges and the Pintwater Range given the close proximities. The microsatellite data yielded exceptionally high values of genetic diversity for the bighorn sheep population in the Sheep, Desert, and East Pahranagat ranges. We speculate that this high diversity likely reflects a long-lived, well connected population. The Sheep Range is a particularly large and high elevation desert mountain range, well connected to neighboring ranges occupied by bighorn sheep. Such systems are more likely to maintain populations through challenges caused by extreme climatic episodes and other variation, such as periods of disease or high predation.

INTRODUCTION

Historically, the Sheep Range in the Desert National Wildlife Refuge (DNWR) contained the largest population of desert bighorn sheep (*Ovis canadensis nelsoni*) in Nevada. Following declines in the mid-1980s, however, population estimates by 1991 had dropped to 18% of what they were six years earlier, and have remained at lower numbers (Nevada Department of Wildlife [NDOW] unpublished data). Declines in bighorn sheep populations can be caused by multiple biotic or abiotic factors, and their interactions (e.g., predation [Wehausen 1996] and disease, [Wehausen et al. 2011]). Specific causes for the decline in the Sheep Range are not definitively known, but several factors have been suggested (see Longshore et al. *in prep.*). A wildlife biologist stationed at DNWR during that time suggested that the initial population decline may have been due to drought conditions; low recruitment rates (12 lambs per 100 ewes in 1988 and 1989) were consistent with this idea (NDOW unpublished data; see Longshore et al. *in prep.*). Such low recruitment, however, could also be consistent with effects of introduced respiratory disease, which can negatively affect bighorn sheep, particularly lamb survival (Wehausen et al. 2011). Another potential explanation followed from radio telemetry data and other observations in the 1990s that indicated mountain lion (*Puma concolor*) predation may have been a significant cause of mortality (see Longshore et al. *in prep.*). These possibilities for the cause of the bighorn sheep population decline, however, were not based on any systematic study and were therefore basically conjectures. The lack of recovery of this population to earlier levels (NDOW unpublished data) certainly suggests that some ecological factor has changed in this ecosystem, and has been the justification to engage in this research to better understand what is driving the current population dynamics.

The initial phase of the overall project, Assessment of Desert Bighorn Sheep on the Desert National Wildlife Refuge, Nevada: Phase 1, encompassed community-level research focused on bighorn sheep and other interacting species in the DNWR, with the intent of providing

information that can be used to formulate an adaptive conservation plan; the actual planning was proposed for a later phase of work. This report summarizes findings of one aspect of the overall initial project involving genetic questions. The main topics concerned questions of genetic diversity and gene flow of bighorn sheep in the Sheep Range relative to bighorn sheep in nearby ranges (Figure 1). A secondary topic concerned genetic diversity and relationships among mountain lions within the Sheep Range.

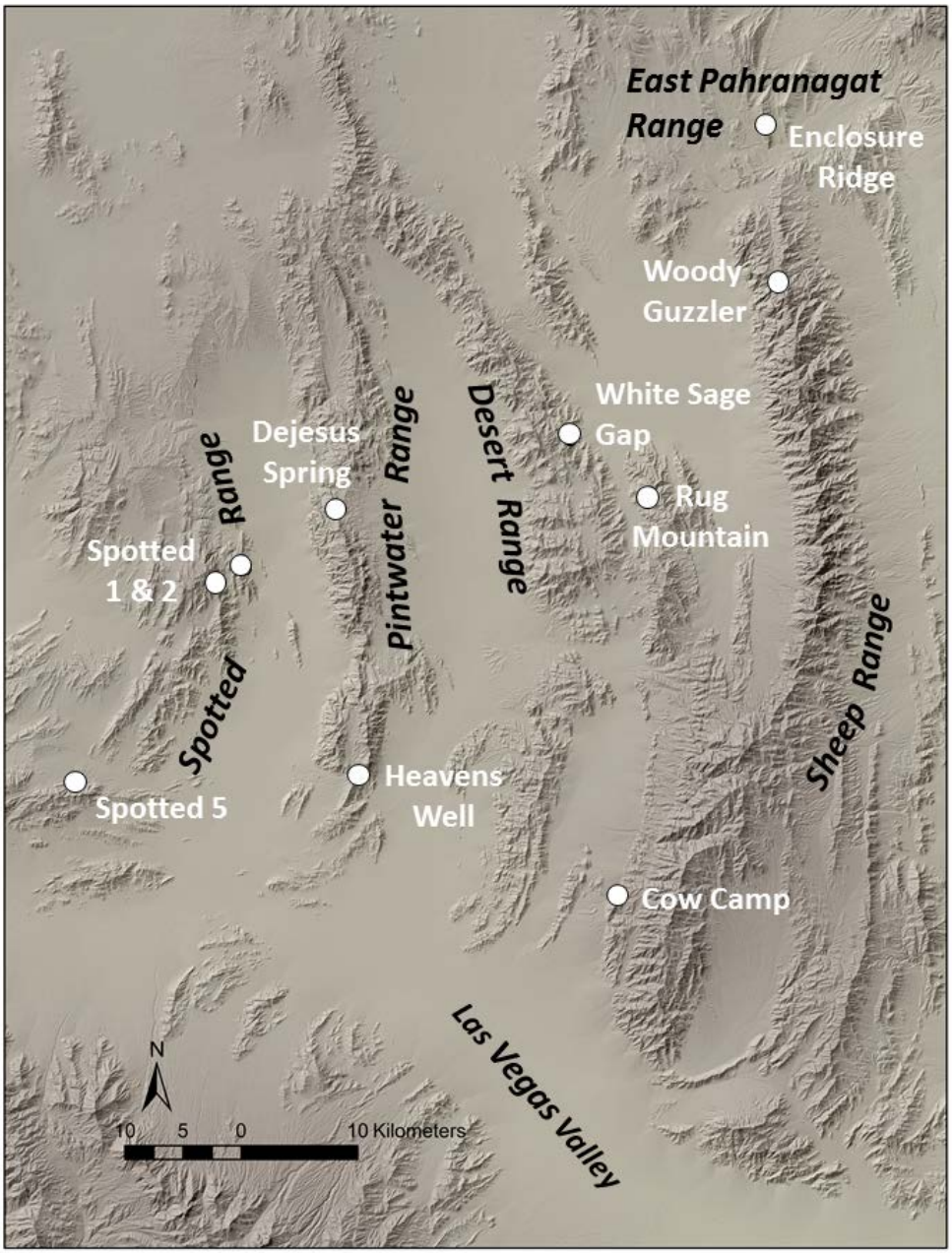

Figure 1. Study site for genetic assessment of bighorn sheep in the area of the Sheep Range, Desert National Wildlife Refuge, Nevada. Shown are water sources where the majority of genetic samples were acquired.

We utilized two types of genetic markers for this research: (1) rapidly evolving, hypervariable nuclear DNA markers known as microsatellites (e.g. Nauta and Weissing 1996; Goldstein and Pollock 1997), which at high levels of resolution allow for individual DNA fingerprinting; and (2) DNA sequence data from the control region (D-loop) of the mitochondria which has been used in previous population assessments of bighorn sheep (e.g., Boyce et al. 1999; Epps et al. 2005; Epps et al. 2007). We conducted the bighorn sheep research in a way that allowed comparisons with a similar project we recently completed for bighorn sheep populations occupying ranges immediately south of the study area (Jaeger and Wehausen 2012). Our efforts were split, with John Wehausen (White Mountain Research Center, University of California) responsible for developing and analyzing microsatellite data, including those used for mountain lions, and Jef Jaeger (University of Nevada, Las Vegas) responsible for control region sequencing and analysis (this latter marker was not used for mountain lions). We shared the tasks of sample collection and DNA extraction.

The two types of genetic markers we used to assess bighorn sheep potentially provide different perspectives of genetic population structure. Bighorn sheep show a high degree of philopatry, especially females, which is manifested in considerable population substructuring (Bleich et al. 1990, 1996). Within larger mountain ranges like the Sheep Range, there are typically multiple subgroups of females that can be defined by different and distinct home range patterns shared by related females. This alone will tend to fragment the genetic population structure, which may be better detected by mitochondrial DNA (mtDNA) that is inherited only from females, thus reflects maternal lineages. In contrast to female bighorn sheep whose life history is focused on rearing young, male bighorn sheep are essentially breeding machines whose behavior leads to genetic influences opposite that of females. Males typically breed outside of their maternal home range, and in so doing maximize nuclear gene flow between the home ranges of maternal subgroups leading toward a more homogeneous mixture of genes across the landscape. Males also commonly move between desert mountain ranges that constitute the core habitat used by bighorn sheep (Bleich et al. 1990, 1996), thus moving genes. How readily bighorn sheep move between ranges is a function of the distance between those ranges and the intervening topography (Epps et al. 2007). As nuclear genetic markers, microsatellites track the effects of gene flow that results substantially from male movements. These markers allow assessments of population level genetic diversity and estimates of gene flow by comparing data for populations using the same loci. Elucidation of genetic population structure can be an important factor for interpreting the dynamics of bighorn sheep populations, as well as for formulating management strategies and actions (e.g., Bleich et al. 1996, Epps et al. 2007).

The Sheep Range has been the recipient of past augmentations of bighorn sheep (NDOW unpublished data), but little is known about the success of those efforts. One method to determine long-term success of translocations is to assess the genetic structure of source populations using detailed genetic analysis and then to determine the contribution of genes from the outside populations into the target populations (e.g. Sheep Range).We applied this method in the context of the genetic structure within the neighboring ranges around the Sheep Range where we expected migration and interchange among populations and subpopulations. Estimates of gene flow from genetic population structure, however, are dependent on the metapopulations sampled

being in approximate equilibrium among population units relative to the dynamics of gene flow. The release of bighorn sheep from outside of the region has the potential to significantly disrupt that equilibrium. Thus, our assessment of the influences of past augmentations on current genetic population structure was a critical precursor to the interpretation of the genetic data produced for this study.

Our genetic research on mountain lions focused on conducting a parentage assessment to confirm and enlighten data from field observations, as well as to provide a description of genetic diversity of mountain lions within the Sheep Range. These assessments first required the development of a panel of microsatellite markers specific for mountain lions, and for this purpose we used DNA from a sample of mountain lions in the Owens Valley region of the eastern Sierra Nevada. The population in the Owens Valley region also allowed us to make meaningful comparisons with the mountain lion sample from the Sheep Range.

METHODS

BIGHORN SHEEP

Study Area and Sample Sources

In the interest of understanding gene flow involving the Sheep Range, this research included the following neighboring habitat patches that supported bighorn sheep populations: Enclosure Ridge in the Pahranagat Range, Desert Range, Pintwater Range, and Spotted Range (Table 1).

Table 1. Sample sites, DNA sources, and numbers of samples acquired for various analyses for bighorn sheep in the Sheep Range and surrounding area of southern Nevada.

Range	Location	DNA Source	Samples Genotyped	Genotypes Obtained	mtDNA Sequences
Sheep	Throughout	blood	30	30	-
Sheep	Cow Camp	feces	24	18	18
Sheep	Woody Guzzler	feces	24	22	19
East Pahranagat	Enclosure Ridge	feces	25	16	16
Desert	White Sage Gap	feces	24	13	13
Desert	Rug Mountain	feces	24	17	17
Pintwater	DeJesus Spring	feces	24	15	15
Pintwater	Heavens Well	feces	24	22	22
Spotted	Guzzler 1	feces	12	7	7
Spotted	Guzzler 2	feces	12	10	10
Spotted	Guzzler 5	feces	21	15	13
TOTALS			244	185	150

We utilized two types of samples as sources of bighorn sheep DNA: blood samples from bighorn sheep captured for the telemetry aspect of the study and fecal samples collected mostly near water

sources used by bighorn sheep. Within mountain ranges, fecal samples were collected at multiple water sources. This allowed us to investigate the potential for genetic substructuring at a finer scale than individual mountain ranges. We set a goal of sampling at least 30 different sheep in each mountain range.

For extraction (isolation) of DNA from fecal material, we required relatively fresh samples collected prior to weathering by rain or excessive exposure to sunlight and heat. Our field sampling was conducted primarily in the hot season when large concentrations of such fecal samples could be found at sites where bighorn sheep drink frequently. We were careful to collect only samples that clearly represented defecation from a single animal and showed no evidence of weathering.

DNA Extraction

Blood samples were collected in tubes containing EDTA. These we centrifuged to harvest the buffy coat (white blood cells) from which we isolated genomic DNA using the Qiagen™ Blood and Tissue DNA Extraction Kit and protocol. To extract DNA from fecal samples, we first carefully scraped the outermost layer from individual fecal pellets (Wehausen et al. 2004). This scraped material provides high concentrations of gut cells from the target animal while minimizing secondary plant compounds that inhibit polymerase chain reaction (PCR) success. During the scraping procedure, we changed gloves and razor blades between samples and cleaned the bench area with a 10% bleach solution. To isolate DNA from the scraped fecal material, we utilized the protocol in Wehausen et al. (2004) but altered it to incorporate an AquaGenomics stool extraction method. For this we used 25-30 mg of scraped outer fecal material, 375 uL of the initial AquaGenomics buffer, and 3.75 uL of 100X proteinase K. The liquid was mixed with the dry fecal scrapings by vortexing and then subjected to bead-beating at high speed for 15 seconds. The resulting foam was converted to liquid by briefly centrifuging the tubes followed by light vortexing to remix, and then incubated for 1 hour at 60-65° C to maximize mtDNA yield.

Microsatellite Data Generation

Microsatellites consist of tandem repeats of small numbers of nucleotides (mostly 2-4) found scattered throughout the genome. While some microsatellites may be linked to genes under selection, most are considered to be selectively neutral which is necessary for investigating gene flow. Microsatellite loci are known as hypervariable genetic markers because they have among the highest mutation rates of any genetic loci studied (Goldstein and Pollock 1997), orders of magnitude higher than loci studied via sequence or allozyme data (Nauta and Weissing 1996). Mutations in microsatellite loci result in different numbers of tandem repeats with each different number of repeats treated as a different allele. By providing more variation (alleles) to work with, the high mutation rates of these markers make them ideal for studies of gene flow.

We developed data for 18 microsatellite loci, all of which are dinucleotide repeats, and most are on different chromosomes (Table 2) thereby maximizing independence. PCRs were formulated as 14 uL reactions, but included an extra 0.7 uL of H_2O to counteract initial dry down. For two of

these loci (MAF36 and TGLA387) we used PCR methods similar to those described in Wehausen et al. (2004) with the loci multiplexed in a single PCR. We amplified the remaining 16 loci in two PCR multiplexes of 8 loci each utilizing the Qiagen Multiplex PCR Master Mix (Qiagen Inc.) at 60° C following the manufacturer's recommendations with variable primer amounts depending on locus strength. We ran PCRs in 96 well plates with each batch of DNA samples including positive and negative controls.

Table 2. Bighorn sheep microsatellite loci used in this study.

Locus	Chromosome	Dye Label	No. of Alleles	Reference
Lane 1				
MAF36	22	tet	8	Swarbrick et al. 1991
OarFCB266	25	6-fam	5	Buchanan and Crawford 1993
HH62	16	hex	14	Ede et al. 1994
MAF209	17	tet	6	Buchanan and Crawford 1992a
OarFCB11	2	6-fam	3	Buchanan and Crawford 1993
OarJMP29	24	tet	10	Crawford et al. 1995
OarFCB304	19	6-fam	5	Buchanan and Crawford 1993
TGLA387	20	hex	6	Georges and Massey 1992
BL4		6-fam	3	Bishop et al. 1994
AE129	5	hex	11	Penty et al. 1993
Lane 2				
AE16	13	hex	7	Penty et al. 1993
OarFCB193	11	6-fam	7	Buchanan and Crawford 1993
MAF65	15	tet	7	Buchanan et al. 1991
MAF33	9	hex	7	Buchanan and Crawford 1992b
MAF48	unknown	6-fam	5	Buchanan et al. 1992
TCRBV62	4	6-fam	8	Crawford et al. 1995
MMP9	13	tet	8	Maddox 2001
OMHC1	20	hex	5	Groth and Weatherall 1994

We electrophoresed PCR products for microsatellite loci on an ABI377 Genetic Analyzer using 96 lanes. For each sample, all 18 loci could be run on two lanes, with different loci in adjacent lanes to eliminate potential bleed over problems. We scored the resulting chromatograms manually using GeneScan 3.1.2 software. For each DNA sample, we ran two replicate PCRs (Wehausen et al. 2004), and when results of replicates exhibited any failures or lack of match, we ran another pair of replicates. Occasionally we had to abandon a sample when it showed clear evidence of poor DNA quality (various problems across multiple loci).

Mitochondrial DNA Sequence Data Generation

Following initial genotyping from microsatellite data, we sequenced most samples identified as unique genotypes for a 515 base pair (bp) portion of the mtDNA control region used in previous

population assessments of bighorn sheep (e.g., Boyce et al. 1999; Epps et al. 2005; Epps et al. 2007; Jaeger and Wehausen 2012). For PCR, we used the previously developed primers Beth (5′ATGGCCCTGAAGAAAGAACC3′) and L15712 (5′AACCTCCCTAAGACTCAAGG3′) in 21 uL reactions using Quiagen Multiplex PCR Master Mix (Quaigen Incorporated) following the manufactures protocol. We generally had good amplifications after 35 thermocycles at an annealing temperature of 60^{o} C (using the recommended conditions on a 2720 Thermal Cycler, Applied Biosystems). We enzymatically cleaned the PCR products prior to sequencing using ExoSAP-IT (USB Corp.).

Our sequencing primers consisted of those used in PCR, but in most cases the Beth primer was replaced by a more internal primer, Beth-Internal-1 (5′ GAT GCC TGT TAA AGT TC 3′; Jaeger and Wehausen 2012). Fluorescence-based cycle sequencing was conducted using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems), with unincorporated dye labels removed by sephadex gel separation. Electrophoresis and visualization was conducted on an ABI 3130 automated sequencer. We aligned sequences using SEQUENCHER v. 4.6 (Gene Codes Corp.), and trimmed the sequences to the targeted stretch of the control region. We then compared sequences to known haplotypes from other studies and identified unique haplotypes using a neighbor-joining assessment based on the number of pairwise differences in MEGA5 (Tamura et al. 2011).

Analyses of Microsatellite Data

The nature of collecting fecal samples may result in multiple samples being inadvertently collected from the same animal. While we used visual attributes of the pellets to minimize the processing of multiple samples from the same animal, we ultimately relied on the microsatellite data to identify and eliminate redundant samples. Because of the high genetic diversity in our samples and the large number of microsatellite loci run, the probability of sampling two different individuals and obtaining identical genotypes (P_{ID}; Waits et al. 2001) was negligible ($\leq 6.5 \times 10^{-6}$ for siblings and lower for unrelated individuals). Overall, 27.6% of the samples we ran were identical to samples already run and were discarded.

We measured population differentiation between pairs of sampling locations (sites or units) using Wright's (1921) F_{ST} measure as implemented in the software FSTAT (Goudet 1995). We used that software to evaluate the statistical significance of a few low F_{ST} values via a randomization test assuming nonrandom mating. We also used FSTAT software to estimate 95% confidence intervals around F_{ST} values via a bootstrapping procedure across loci. We used F_{ST} values to calculate estimates of gene flow (Nm) using Wright's (1921) equation $F_{ST} = 1/(1+4Nm)$ to derive the following equation to estimate gene flow: $Nm = (1- F_{ST})/4* F_{ST}$. We used the 95% confidence intervals around F_{ST} values to estimate such intervals for Nm. We used the F_{ST} results as one basis for deciding which sample locations should be pooled where it was not justified to treat them as separate populations.

Of the ranges that we sampled, both the Sheep and Spotted Ranges were the recipients of bighorn sheep translocated in the 1990s (Table 3). In 1996, 5 bighorn sheep from the Eastern Pahranagat

Range were moved to the middle of the Sheep Range. A more substantial translocation occurred in 1998, when 35 bighorn sheep were released in the south Sheep Range from 3 sources: the Muddy Mountains (n = 10), the Arrow Canyon Range (n = 10), and the Specter Range (n = 15). The Specter Range itself had received a considerable infusion of sheep (n = 44) from the Muddy and River Mountains earlier that decade (Table 3). In contrast to the Sheep Range, the Spotted Range received 50 bighorn sheep in the 1990s from only a single source, the River Mountains (Table 3). Of those different sources of sheep, we had already generated genetic data for the Muddy and River Mountains.

Table 3. Translocations of bighorn sheep in Nevada pertinent to this study (NDOW unpublished data).

Year	To	From	Sheep Moved
1990	Specter Range	River Mountains	9
1990	Specter Range	Muddy Mountains	10
1993	Specter Range	Muddy Mountains	20
1995	Specter Range	River Mountains	5
1993	Spotted Range	River Mountains	25
1996	Spotted Range	River Mountains	25
1996	Middle Sheep Range	East Pahranagat Range	5
1998	South Sheep Range	Muddy Mountains	10
1998	South Sheep Range	Arrow Canyon Range	10
1998	South Sheep Range	Specter Range	15

We used the software STRUCTURE (Pritchard et al. 2000) to test for the influence of translocations from the Muddy and River Mountains on current genetic population structure in the study area. Because our previous data on the Muddy and River Mountains lacked two of the microsatellite loci run for the current study, this test was based on the 16 loci run in both studies. We included 34 samples from the Muddy Mountains and 46 samples from the River Mountains, and also included the data from the blood samples collected in the Sheep Range. In this test, we varied the number of clusters (K) and examined the probabilities of assignments of individuals to the different clusters and the average of those probabilities for sampling locations. For each STRUCTURE run we used 250,000 iterations with the first 50,000 discarded as burnin.

We also conducted a second STRUCTURE analysis to explore overall population structure represented in our data only from the study area, including the Sheep Range blood samples; thus, in contrast to the previous analysis, we did not include data from the Muddy and River Mountains. In this analysis, we conducted independent runs for 2 to 8 specified numbers of clusters (K). We replicated each of these runs 20 times to produce the needed output variance to use the method recommended by Evanno et al. (2005) for analysis of the output results. For each replicate run, we used 100,000 iterations discarding the initial 20,000 as burnin.

We used the software NEESTIMATOR (Peel et al. 2004) to estimate breeding effective population size (N_e) for each site or sampling unit using the single sample linkage disequilibrium method. We developed the following measures of genetic diversity using the software POPGENE (available

at www.ualberta.ca/~fyeh/index.htm): average number of alleles per locus; effective number of alleles (which is the number of alleles at equal frequency that would give the measured homozygosity; Hartl and Clark 1997); observed heterozygosity and expected heterozygosity (corrected for sample size; Nei 1987). Using the software FSTAT, we calculated allelic richness as the average number of alleles per locus adjusted to the lowest sample size (Leberg 2002).

Analyses of Mitochondrial DNA Haplotypes

We conducted analyses of mtDNA control region sequences using haplotype frequencies at sample sites or units. We calculated pairwise F_{ST} measures and estimated gene flow (Nm = number of migrants) using the software ARLEQUIN 3.11 (Excoffier et al. 2005), with the significance of these indices determined by 1000 non-parametric permutations. Estimates of Nm from haploid data were in essence measures of female migration, and therefore were not on the same scale as those derived from microsatellite data, as described above. We also used ARLEQUIN to calculate standard genetic diversity indices. These measures included haplotype diversity (Nei 1987) which incorporates sample size and the frequency of each haplotype in the sample, and nucleotide diversity using pairwise differences which is a measure of the genetic difference between samples (Tajima 1993).

MOUNTAIN LIONS

Microsatellite Data Development

We extracted DNA from blood samples collected from mountain lions captured in the Sheep Range using the methods described above for bighorn sheep blood. We also extracted DNA from blood or tissue samples from 18 mountain lions from the eastern Sierra Nevada in the Owens Valley region. We developed a panel of 19 microsatellite loci (Table 4) that could be run in two lanes and amplified in four PCRs, based on loci known to have variation in mountain lions (Ernest 2001, Menotti-Raymond and O'Brien 1995, Culver et al. 2001, Kurushima et al. 2006). The Owens Valley samples were used to refine our protocols for this panel, and for some loci the reverse primer was lengthened with a "tail" to increase the spread among loci during electrophoreses.

We used PCR conditions that were similar to those described above for bighorn sheep. Sixteen loci were amplified in two PCR multiplexes of eight each, utilizing the Qiagen Multiplex PCR Master Mix with annealing at 60° C following manufacturer recommendations. Two replicate PCRs were run for each DNA sample. We varied primer amounts depending on locus strength. Three loci (Fca35, Fca78, and Fca126; Table 4) were amplified separately using PCR conditions similar to those described in Wehausen et al. (2004) with Fca78 and Fca126 multiplexed in a single PCR. Each batch of samples included positive and negative controls. All PCR products for microsatellite loci were electrophoresed on an ABI377 Genetic Analyzer using 96 lanes, with different loci in adjacent lanes to eliminate potential bleed over problems. We scored the resulting chromatograms manually using GeneScan 3.1.2 software.

Microsatellite Data Analyses

We applied the same genetic diversity measures used for bighorn sheep to the mountain lion data. Field information indicated that the lions captured in the Sheep Range were likely closely related (see Results and Discussion). To confirm field observations, we looked at the genetic relationships between these lions as the proportion of all alleles that were shared between individuals. We used the software GIMLET (Valière 2002) to determine pairs of samples that would be consistent with parent-offspring relationships; such pairs should share at least one allele for every locus. This differs from full-siblings which on average share 50% of their alleles, but do not necessarily share one for every locus.

Table 4. Mountain lion microsatellite loci used in this study.

Locus	Dye	Tail length	Source
Lane 1			
PcoC108w	hex		Kurushima et al. (2006)
Fca43	6-fam		Menotti-Raymond and O'Brien 1995
Fca126	tet	5 basepairs	Menotti-Raymond and O'Brien 1995
Fca8	hex		Menotti-Raymond and O'Brien 1995
Fca132	6-fam		Menotti-Raymond et al. 1999
PcoB210w	hex		Kurushima et al. (2006)
Fca78	tet		Menotti-Raymond and O'Brien 1995
Fca96	hex		Menotti-Raymond and O'Brien 1995
Fca166	6-fam		Culver et al. 2001
Fca82	6-fam		Menotti-Raymond et al. 1999
Lane 2			
Fca90	6-fam		Menotti-Raymond and O'Brien 1995
Fca35	tet		Menotti-Raymond and O'Brien 1995
Fca77	hex		Menotti-Raymond and O'Brien 1995
Fca26	hex	4 basepairs	Menotti-Raymond et al. 1999
PcoC112	tet	7 basepairs	Kurushima et al. (2006)
Fca57	6-fam		Menotti-Raymond et al. 1999
PcoA208w	6-fam	7 basepairs	Kurushima et al. (2006)
PcoB010w	tet		Kurushima et al. (2006)
PcoC217w	hex		Kurushima et al. (2006)

RESULTS AND DISCUSSION

BIGHORN SHEEP – MICROSATELLITE DATA

Genotypes

We genotyped a total of 244 samples which yielded 185 different genotypes and met our goal of at least 30 genotypes per mountain range (Table 1). The number of genotypes from any particular

sample site ranged from 7 to 22, and we also developed 30 genotypes from the blood samples collected from locations throughout the Sheep Range.

Population Differentiation

The degree of population differentiation is a function of gene flow (Nm) which we estimated from F_{ST} values. The relationship between F_{ST} and Nm is a hyperbolic curve of which only a central portion, where the curve transitions between its two asymptotic arms, yields seemingly meaningful variation in Nm at F_{ST} values between approximately 0.025 and 0.15. Very high levels of gene flow are reflected by F_{ST} values less than ~ 0.025. Differences in gene flow at this range of values cannot be resolved by Nm estimates from F_{ST}; instead such values are consistent with a lack of population differentiation. Values of F_{ST} greater then ~ 0.15 also cannot be resolved and reflect essentially no gene flow.

Table 5. F_{ST} data for sample sites and units lacking statistical significance, or with very high gene flow that did not justify retaining them as separate sample units. Also shown are bootstrapped 95% confidence intervals. Abbreviations for ranges: SH=Sheep Range, DR=Desert Range, EPR=East Pahranagat Range, SP=Spotted Range, RI = River Mountains. Abbreviations for sites: CC=Cow Camp, ER=Enclosure Ridge, HW=Heavens Well, RM=Rug Mountain, WG=Woody Guzzler, WS=White Sage.

Sample 1		Sample 2			95% CI	
Range	**location**	**Range**	**location**	**F_{ST}**	**L**	**H**
SH	WG	EPR	ER	-0.002	-0.009	0.006
DR	WS	DR	RM	0.016	0.000	0.034
SP	Guzzler 1	SP	Guzzler 2	0.014	-0.009	0.038
SH	CC	DR	WS+RM	0.026	0.013	0.041
SH	blood	SH	WG+ER	0.009	0.003	0.015
SH	blood	SH, DR	CC+DR	0.008	0.001	0.015
SP	Guzzlers 1, 2, & 5	RI	range wide	0.015	0.007	0.023

We found three pairs of geographically closely situated sites that lacked significant F_{ST} values, exhibiting 95% confidence intervals that included zero: Woody Guzzler and Enclosure Ridge, White Sage Gap and Rug Mountain, and Spotted Range Guzzlers 1 and 2 (Table 5). The Spotted Range Guzzlers 1 and 2 are located only 2.7 km apart. Similarly, Rug Mountain and White Sage Gap within the Desert Range are separated by only 8.4 km of relatively continuous mountainous terrain. Woody Guzzler and Enclosure Ridge are further apart at 13.4 km, although mountainous terrain mostly connects these sites in the northern portion of the Sheep Range and adjacent East Pahranagat Range. We combined data from each of these pairs of sites to form sample units and did not tabulate the original sites separately in subsequent analyses. The resulting combined unit of White Sage Gap and Rug Mountain (Desert Range), when assessed with Cow Camp in the southern Spring Range, had an F_{ST} = 0.026 and an estimated Nm of 9.4 migrants per generation. While exhibiting an F_{ST} value that was significantly ($P \leq 0.05$) different from zero, we considered this F_{ST} value also outside the range of effective resolution as described above. We therefore also combined the samples from these sites to form a sampling unit representing the southern portion

of the Sheep Range that included the Desert Range. This decision was supported by telemetry data showing that some bighorn sheep collared in the Sheep Range made extensive use of the southern end of the Desert Range.

The blood samples obtained during capture events were collected from locations throughout the Sheep Range. We combined these samples to represent the Sheep Range in general; however, we found this to be a geographically crude representation of the Sheep Range when compared to the localized sites associated with the fecal sample collections. The combined blood samples from the Sheep Range, when compared with each of the different geographic subsamples from sites within the Sheep and Desert ranges, had F_{ST} values ≤ 0.026 (Table 5). Thus, the combining of blood samples was not useful relative to the larger question of population differentiation. While we could have parsed the blood samples geographically using telemetry data on home ranges of the sheep sampled, for the question of population differentiation, we found our data from the fecal collections to be sufficient for most analyses.

Table 6. F_{ST} data for defensibly different sampling units from neighboring mountain ranges with bootstrapped 95% confidence intervals and gene flow (Nm; migrants per generation) estimates. Abbreviations for ranges: SH=Sheep Range, DR=Desert Range, EPR=East Pahranagat Range, PW=Pintwater, SP=Spotted Range. Abbreviations for sites: CC=Cow Camp, ER=Enclosure Ridge, HW=Heavens Well, RM=Rug Mountain, WG=Woody Guzzler, WS=White Sage. For F_{ST} values below 0.025 Nm, estimates were simply listed as high.

Sample 1		**Sample 2**			**95% CI**			**95% CI**	
Range	**Location**	**Range**	**Location**	**F_{ST}**	**L**	**H**	**Nm**	**L**	**H**
SH+EPR	WG+ER	SH,DR	CC+DR	0.037	0.023	0.052	6.5	4.6	high
PW	HW	PW	DS	0.051	0.021	0.082	4.6	2.8	high
SP	Guz. 1+2	SP	Guz. 5	0.056	0.028	0.084	4.2	2.7	8.7
PW	HW	SH,DR	CC+DR	0.086	0.057	0.115	2.7	1.9	4.1
PW	DS	SH,DR	CC+DR	0.060	0.032	0.09	3.9	2.5	7.6
PW	HW	SH+EPR	WG+ER	0.082	0.048	0.119	2.8	1.8	5.0
PW	DS	SH+EPR	WG+ER	0.078	0.042	0.118	3.0	1.9	5.7
PW	HW	SP	Guz. 1+2	0.140	0.093	0.191	-	-	-
PW	DS	SP	Guz. 1+2	0.160	0.112	0.211	-	-	-
PW	HW	SP	Guz. 5	0.134	0.078	0.191	-	-	-
PW	DS	SP	Guz. 5	0.118	0.073	0.167	-	-	-

The result of combining the data from the sites discussed above was a final data set representing six geographically separate units that showed some meaningful level of genetic population structure, two each in three mountain masses (Table 6). Prior to estimating gene flow amongst the six sample units, however, it was necessary to assess the influence of translocations from the Muddy and River Mountains (Table 3) on population genetic structure within the study area.

Influences of Translocations on Genetic Population Structure

Our STRUCTURE analysis to evaluate the influence of translocations on population structure exhibited a consistent strong assignment of samples from the Spotted Range with samples from the River Mountains. In contrast, there was a lack of assignment of samples from the Sheep and Desert ranges with samples from either the Muddy or River Mountains. When we forced samples into two clusters, samples from the Muddy Mountains, River Mountains, and Spotted Range made up one cluster, with the remaining samples from all other ranges in the other cluster (Table 7). When we allowed three clusters, samples from the Pintwater Range emerged as an independent cluster. At four clusters, samples from the Muddy Mountains formed their own cluster (Table 7). Increasing cluster numbers beyond this did not separate the Spotted and River samples. The strong influence of past translocations from the River Mountains on the genetic structure in the Spotted Range was clearly evident, and was corroborated by a very low F_{ST} value for those ranges (Table 5).

Table 7. Clustering patterns for various numbers of assigned clusters (K) from STRUCTURE analysis (whole numbers reference clusters) and average assignment probabilities for sampling units to their primary cluster.

K	Sheep & Desert	Pintwater	Muddy	River	Spotted
2	1 (0.955)	1 (0.955)	2 (0.988)	2 (0.967)	2 (0.985)
3	1 (0.907)	2 (0.933)	3 (0.975)	3 (0.947)	3 (0.975)
4	1 (0.766)	2 (0.920)	3 (0.967)	4 (0.947)	4 (0.837)

As we increased cluster numbers, a decline in average assignment probabilities for samples from the greater Sheep Range (Sheep, Desert and East Pahranagat ranges) was evident (Table 7). The decline was made up by the increased assignment probabilities of samples from the eastern portion of the study area (greater Sheep Range) to the Pintwater cluster and not to the River or Muddy mountains clusters. At four clusters (K=4), samples from the greater Sheep Range only had 0.017 probabilities of being assigned to the River or Muddy Mountains clusters.

Because the 1998 translocations were to the southern Sheep Range, we reran this analysis with only the samples from Cow Camp and Desert Range representing the southern Sheep Range, which also provided a much more equal representation of samples from the various geographic regions. Under this analysis for three clusters, samples from the Muddy Mountains, rather than the Pintwater Range, emerged as the third cluster, leaving the samples from the Pintwater Range clustered with samples from further east. At four clusters, samples from the Pintwater Range emerged as a separate cluster, but assignment of samples from the Pintwater, southern Sheep and Desert ranges to the cluster of samples from the Muddy Mountains remained negligible. Again, there was negligible assignment to the River Mountains cluster of samples from the southern Sheep and Desert ranges, while assignment to the River Mountains was slightly elevated for samples from the Pintwater Range. Examination of assignment values for individual Pintwater Range samples indicated that the slightly elevated assignments to the River Mountains cluster was substantially driven by what appeared to be two f1 hybrids between the Pintwater and Spotted Ranges. Similar hybrids were not evident among the samples in the Spotted Range. In

short, our analyses failed to find genetic evidence from microsatellite data for the infusion of Muddy and River Mountains sheep into the Sheep Range from the translocations.

Gene Flow Estimates within Ranges

We tabulated defensible gene flow estimates between adjacent sampling units retained in the Sheep, Desert, Pintwater, and Spotted ranges (Table 6). For pairs of sampling units, the highest estimate of gene flow was between the units representing the two ends of the Sheep Range, with the southern end including the Desert Range, followed by between the two units in the Pintwater Range, and then between the two units in the Spotted Range. All of these estimates essentially represented comparisons of samplings within mountain ranges and reflected relatively high gene flow levels that were consistent with low population structure, and justified combining those sample pairs for estimates of genetic diversity and effective population size (see below).

Gene Flow Estimates between Ranges

Estimated gene flow between each sample unit within the Pintwater Range and those in the Sheep and Desert ranges were lower than between unit pairs within each of these ranges (Table 6). While still reflecting substantial amounts of gene flow, these values suggest a higher level of population structure between ranges compared to within ranges, as would be expected. We did not calculate estimated gene flows between the Pintwater Range and the adjacent Spotted Range because of the strong influence of translocations on the genetic population structure in the Spotted Range. A basic assumption of such gene flow estimates is that the metapopulation in question has reached equilibrium. This will take considerably more time than the couple of generations since the 50 bighorn sheep were translocated to the Spotted Range. A number of the F_{ST} values and their confidence limit values for sampling units in the Spotted and Pintwater ranges lie near and beyond the upper limit ($F_{ST} = 0.15$) of where there is any ability to resolve differences in gene flows (Table 6). Those estimates would simply be characterized as very low and potentially undetectable gene flows such as we have found previously for samples across water bodies further south in Nevada (Jaeger and Wehausen 2012). Given the relatively small distances between the sample units in the Spotted and Pintwater ranges, considerable gene flow would be expected and probably is occurring; however, we were unable to validly estimate its magnitude because of the translocations. Recent gene flow between these ranges was evidenced by the apparent f1 hybrids between the gene pools in these two ranges, as noted above.

Assessment of Population Structure based on STRUCTURE Analysis

The results from our second STRUCTURE analysis to assess population structure represented in the data only from the study area were consistent with our earlier estimates of gene flow from F_{ST} values. The greatest support was for three clusters which by the analysis of Evanno et al. (2005) most likely represents the true population structure. The three clusters were the Spotted Range, Pintwater Range, and the combination of all samples from the Desert, Sheep and East Pahranagat ranges further east (Figure 2).

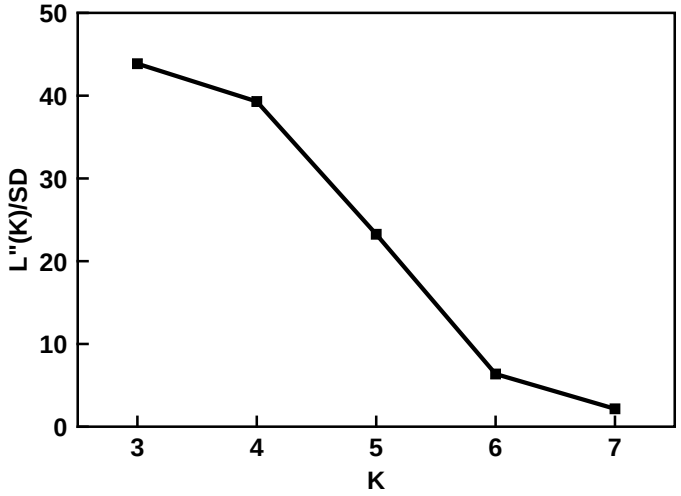

Figure 2. Results of STRUCTURE analyses of data from 18 microsatellite loci for samples from the Spotted, Pintwater, Desert, Sheep, and East Pahranagat ranges in southern Nevada following the methods of Evanno et al. (2005).

Table 8. Bighorn sheep genetic diversity measures: average number of alleles per locus (A), allelic richness (AR), effective number of alleles per locus (A_E), observed heterozygosity (H_O), expected heterozygosity (H_E), and genetically effective population size (N_E) estimates. N = sample size.

Range(s)	N	A	AR	A_E	H_O	H_E	N_E (95% CI)
18 loci							
Sheep & Desert & Enclosure	116	6.6	5.8	3.9	0.695	0.713	86 (77-96)
Pintwater	37	5.3	5.1	3.2	0.628	0.650	40 (33-48)
Spotted	33	4.2	4.2	2.9	0.615	0.626	22 (19-27)
16 loci							
Sheep & Desert & Enclosure	116	7.0	6.2	4.0	0.708	0.724	96 (85-109)
Pintwater	37	5.4	5.3	3.3	0.650	0.671	41 (34-52)
Spotted	33	4.2	4.2	3.0	0.612	0.633	20 (17-25)
Muddy	34	4.4	4.7	2.8	0.612	0.585	54 (40-79)
River	46	4.9	4.3	2.9	0.637	0.630	127 (84-238)
Eldorado	60	5.4	5.0	3.3	0.672	0.680	60 (50-73)
McCullough & Highland	64	5.3	5.2	3.1	0.673	0.670	94 (74-126)

Genetic Diversity and Effective Population Sizes

For estimates of genetic diversity and effective population size, we used the three clusters supported by our STRUCTURE analysis, including data from blood samples for the Sheep Range. For these measures, we only used 16 of our loci to allow inclusion of comparative data from ranges previously sampled further south in Nevada (data from Jaeger and Wehausen 2012). For our four measures of genetic diversity, the combined sample from the Sheep, Desert, and East Pahranagat ranges stands out as having the highest values we have recorded, with more than one allele per locus higher than the next closest value (Table 8). Values for the Pintwater Range were

similar to populations further south, while the Spotted Range falls slightly lower. Estimates of genetically effective population sizes follow the same relative pattern among the three genetic units (Table 8).

BIGHORN SHEEP – MITOCHONDRIAL DATA

Sequences, Haplotypes, and Genetic Diversity

Of the 155 individuals that we identified by genotyping using microsatellite data from samples collected at water sources (sites), we successfully sequenced 150 for mtDNA control region (Table 1). We identified six haplotypes within the study area (Figure 3). When sites were combined within ranges, the Sheep Range contained 4 haplotypes, the Desert and Pintwater ranges both contained 3 haplotypes, and the Spotted Range contained 2 haplotypes (Table 9). In ranges further to the south, the average number of haplotypes per range was 2.9 with maxima of 5 haplotypes found in well-connected ranges (Jaeger and Wehausen 2012). In the eastern Mojave Desert, the average number of haplotypes per population was 2.75 with a maximum of 6 haplotypes among 24 populations assessed (excluding several known to be newly established by colonization; Epps et al. 2010). The low haplotype diversity in the Spotted Range appears to have resulted from the historical sparsity of bighorn sheep in the range and the subsequent translocations into the range of sheep with limited mtDNA diversity (see below).

Table 9. Diversity statistics for mtDNA control region sequence data by sample sites and mountain ranges. Standard deviations are provided for the measures of diversity. Abbreviations for sites: CC=Cow Camp, ER=Enclosure Ridge (East Pahranagat Range), HW=Heavens Well, RM=Rug Mountain, WG=Woody Guzzler, WS=White Sage.

Sample Area	Sample Size (Haplotypes)	Haplotype Diversity	Nucleotide Diversity	Pairwise Differences
Sheep Range CC+WG+ER	53 (4)	0.678 ± 0.0357	0.009 ± 0.005	4.749 ± 2.360
Sheep Range southern, CC	18 (3)	0.451 ± 0.117	0.004 ± 0.003	2.248 ± 1.297
Sheep Range northern, WG+ER	35 (4)	0.504 ± 0.089	0.007 ± 0.004	3.590 ± 1.867
Desert Range, WS+RM	30 (3)	0.248 ± 0.010	0.004 ± 0.003	2.032 ± 1.175
Pintwater Range, DS+HW	37 (3)	0.589 ± 0.050	0.006 ± 0.004	3.147 ± 1.668
Pintwater Range, DS	15 (2)	0.419 ± 0.113	0.007 ± 0.004	3.352 ± 1.821
Pintwater Range, HW	22 (2)	0.173 ± 0.101	0.001 ± 0.001	0.693 ± 0.545
Spotted Range, Guzzlers 1+2+5	30 (2)	0.239 ± 0.092	0.006 ± 0.003	2.869 ± 1.552
Spotted Range, Guzzlers 1+2	17 (1)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Spotted Range, Guzzler 5	13 (2)	0.462 ± 0.110	0.011 ± 0.006	5.538 ± 2.846

Of the 6 haplotypes found in the study area, 2 (E and M) matched previously identified haplotypes from the Mojave Desert (Epps et al. 2005), and we also previously found these haplotypes in ranges to the south of the current study area (Jaeger and Wehausen 2012). Another of the haplotypes (D2) was previously identified from the Black Mountains of Arizona on the

eastern side of the Colorado River where it appeared to be fixed in that population (Jaeger and Wehausen 2012). Interestingly, haplotype D2 had not been previously found on the west side of the river, except in a sample that likely resulted from a sample mix-up (Jaeger and Wehausen 2012); yet, we found it in high frequency in the northern portion of the Sheep Range and in the East Pahranagat Range. We identified three new haplotypes within the study area (DR, SH, and S2). Haplotype SH was most common and wide spread, while haplotype DR was the least common and only observed in two sheep from Rug Mountain in the Desert Range (Figure 3).

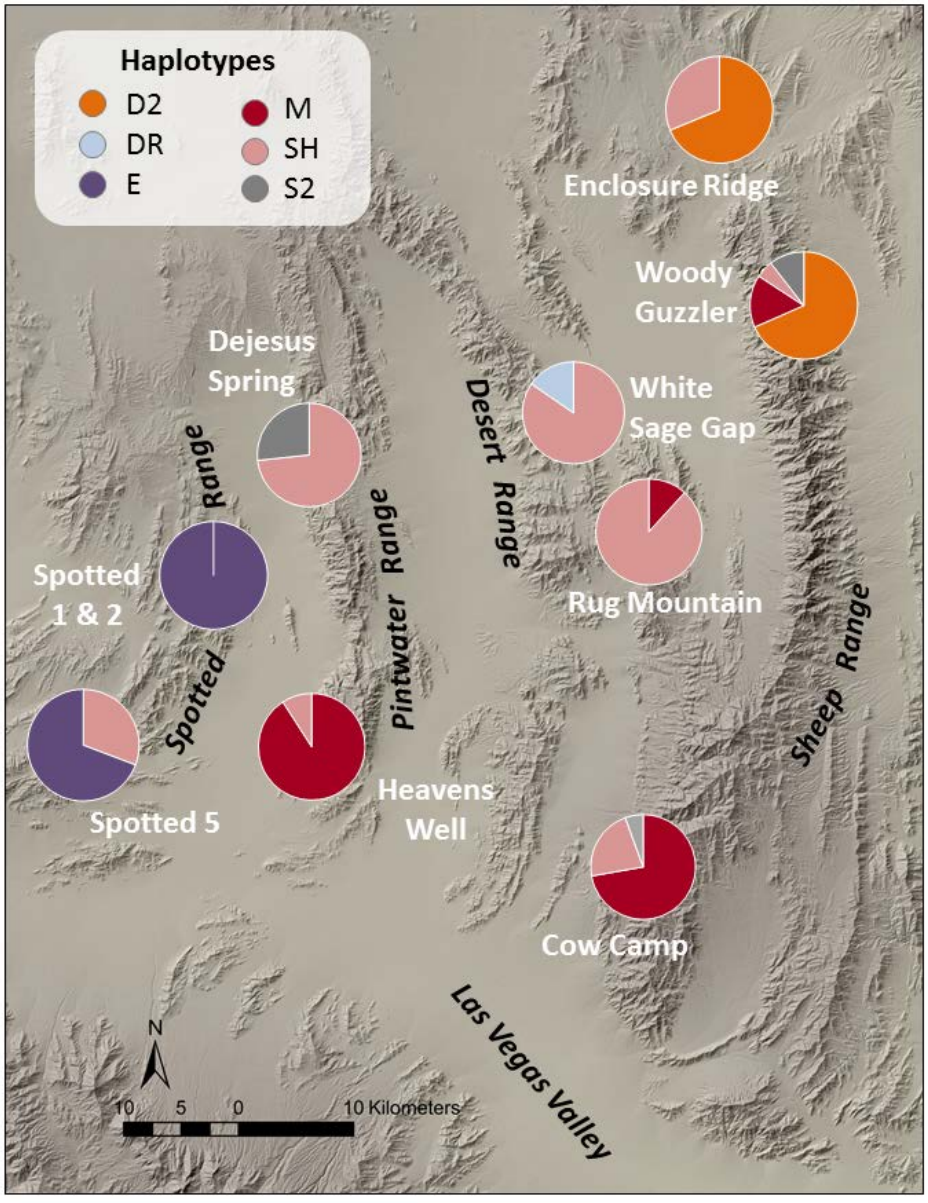

Figure 3. Haplotype distribution and frequency of mtDNA control region sequences from 150 bighorn sheep in the area of the Sheep Range, Nevada. Pie colors reference haplotypes. The sizes of pie slices indicate the frequencies of haplotypes at each location. Samples from Guzzlers 1 and 2 in the Spotted Range are combined. Shading relief represents topography with the major mountain ranges and the northern-end of the Las Vegas Valley indicated.

Influences of Translocations on Genetic Population Structure

The mtDNA data were largely consistent with microsatellite data in terms of the strong impact on the genetic structure in the Spotted Range of translocations from the River Mountains (Table 3). The most common mtDNA haplotype we detected in the Spotted Range (haplotype E) was not found in any of the other ranges to the east of the Spotted Range (Figure 3). This haplotype was the only one detected in River Mountains (Jaeger and Wehausen 2012) and therefore would have been highly represented in the animals released to the Spotted Range. A few of the individuals we sampled in the southern Spotted Range, however, had a haplotype that was common in the ranges further to the east (haplotype SH; Figure 3) and this haplotype was not found in the River Mountains or other ranges further south (Jaeger and Wehausen 2012). The two most likely explanations for the presence of this haplotype in the Spotted Range was that a few native females were present when the introductions occurred, or our sample represents rams that recently migrated into the range. We did not assess the sex of our samples and so cannot differentiate between these two possibilities.

In both the southern Sheep Range (at Cow Camp) and in the southern Pintwater Range (at Heavens Well), we observed curiously high percentages of haplotype M (Figure 3). In our previous study, we found that this haplotype was common across ranges further south and nearly fixed in the Muddy Mountains (Jaeger and Wehausen 2012). As a consequence, haplotype M was probably highly represented in the translocations to the southern Sheep Range derived from both the Muddy Mountains and Spector Range (Table 3). The high percentage of this haplotype in the southern Sheep Range might be interpreted as indicating a substantial influence of these translocations, contrary to our interpretation from microsatellite data. The Muddy Mountains, however, is the next major mountain range to the southeast of Arrow Canyon Range, which is located directly east of the southern Spring Range. There are broken badlands and ridges that provide potential pathways by which female bighorn sheep may have naturally migrated between these ranges. To complicate interpretations further, however, the Arrow Canyon Range was also used as a source of bighorn sheep for a translocation to the southern Sheep Range (Table 3). The potential interpretation of our data that haplotype M within the study area was a natural occurrence was supported by the high frequency of this haplotype in the southern Pintwater Range far from the translocation sites.

Population Differentiation

Pairwise estimates of divergence from mtDNA sequence data were consistent with microsatellite data showing very low levels of genetic structure within three sets of geographically closely situated sites. We detected no significant difference in genetic structure based on haplotype frequencies between (1) Spotted Range Guzzlers 1 and 2 (F_{ST} = 0.000; P = 0.999), (2) Rug Mountain and White Sage Gap within the Desert Range (F_{ST} = 0.072; P = 0.193), and (3) Woody Guzzler in the Sheep Range and Enclosure Ridge to the north in the East Pahranagat Range (F_{ST} = 0.012; P = 0.297). These sites lack justification for being retained as separate samples, and we combined data from the respective pairs of sites in subsequent assessments. Unlike the microsatellite data (see above), the mtDNA data showed a relatively high level of divergence

between Cow Camp in the southern Spring Range and the Desert Range (Table 10), and for mtDNA analyses we retained these regions as separate sample units. Conversely, Cow Camp in the southern Sheep Range and Heavens Well in the Pintwater Range showed essentially no genetic difference (Table 9), even though more than 24 km separate these sites, including large areas of flat desert (Figure 3). We retained these sites as separate sample units given the lack of direct habitat connectivity. Because of the influence of translocations on the Spotted Range, as described above, we did not calculate pairwise estimates of the number of migrants (Nm).

Table 10. Pairwise F_{ST} and associated Nm (gene flow) estimates derived from mtDNA control region sequence data for adjacent sites or mountain range pairs. Asterisks indicated samples pairwise F_{ST} values that were not significantly different from zero ($P > 0.05$). Site identifications: CC=Cow Camp, DS=Dejesus Spring, HW=Heavens Well, ER=Enclosure Ridge, WG=Woody Guzzler, WS=White Sage, RM=Rug Mountain.

Sample 1	Sample 2	F_{ST}	Nm
Sheep Range southern, CC	Sheep Range northern, WG+ER	0.528	0.581
Sheep Range southern, CC	Desert Range, WS+RM	0.442	0.392
Sheep Range southern, CC	Pintwater Range, HW	0.037*	11.80
Sheep Range southern, CC	Pintwater Range, DS	0.377	0.564
Sheep Range northern, WG+ER	Desert Range, WS+RM	0.559	0.412
Desert Range, WS+RM	Pintwater Range, DS	0.091*	4.509
Pintwater Range, DS	Spotted Range, Guzzlers 1+2	0.856	-
Pintwater Range, HW	Spotted Range, Guzzlers 1+2	0.967	-
Pintwater Range, HW	Spotted Range, Guzzler 5	0.687	-
Spotted Range, SP1+SP2	Spotted Range, Guzzler 5	0.291	-

MOUNTAIN LIONS

Five lions were caught in the Sheep Range, four females and one male. Field information indicated that two of the females (F002 and F004) were offspring of another female (F001). The fourth female (F005) was somewhat older than F001. The male (M003) was a juvenile at dispersal age that ultimately traveled a considerable distance from the range. The proportion of alleles shared between individual lions from the Sheep Range varied from a low of 0.474 between F004 and F005, to a high of 0.789 between F001 and F002. Results of parentage analysis suggest that most of the lions captured during this study represented three generations of one matriline. That analysis identified F002 and F004 as offspring of F001 as expected, but also identified F005 as the mother of F001. These two lions shared 71% of their alleles. M003 was the only lion that was not identified as an offspring or a parent within the group; however, his proportion of shared alleles with F001's daughter F004 (0.684) was higher than between that mother-daughter pair (0.668). He also shared a high proportion (0.632) of alleles with F001. These results suggest that the mother of M003 was another close relative in this matriline.

Because only five mountain lions were caught during this study, our analyses were substantially descriptive and we provided comparisons with data from the Owens Valley region. These two samplings differed considerably in allele frequencies resulting in a significant F_{ST} value of 0.130 (95% CI: 0.066-0.192). The five lions from the Sheep Range showed lower genetic diversity compared with the sample of lions from the Owens Valley, as would be expected given the close relationship between the Sheep Range lions. The Owens Valley samples had variation in all 19 loci whereas the Sheep Range sample showed no variation in three loci. As would be expected for the small sample size from the Sheep Range, there were considerably fewer alleles sampled there (n = 41) compared with the Owens Valley (n = 65). This is similarly reflected in differences in the average number of alleles per locus (Table 11). Despite lower genetic diversity in the Sheep Range, there were two private alleles not sampled in the Owens Valley. Sample size alone does not explain these differences in allele numbers. When the two samples were equalized as allelic richness values by adjusting them to the sample size from the Sheep Range, the Owens Valley sample continued to show notably greater genetic diversity, which was also reflected in heterozygosity level differences and N_E estimates (Table 11).

Table 11. Mountain lion genetic diversity measures for 19 microsatellite loci: average number of alleles per locus (A), allelic richness (AR), effective number of alleles per locus (A_E), observed heterozygosity (H_O), expected heterozygosity (H_E), and genetically effective population size (N_E) estimates. N = sample size.

Population	**N**	**A**	**AR**	**A_E**	**H_O**	**H_E**	**N_E (95%CI)**
Owens Valley Region	18	3.4	2.7	2.6	0.579	0.587	15.0 (12.2-18.9)
Sheep Range	5	2.2	2.0	1.9	0.453	0.402	2.9 (2.2-3.9)

CONCLUSIONS

MOUNTAIN LIONS

As a relatively high elevation mountain range with associated habitat variation, the Sheep Range, along with nearby mountain ranges, support a greater density and diversity of prey than many areas of the Mojave Desert. Large mammals constitute the main prey base for mountain lions, and the Sheep Range system includes both mule deer and bighorn sheep. The greater prey base currently allows this region to support a resident mountain lion population. The habitat, however, appears to be relatively marginal for mountain lions, and this marginality is reflected in the data on genetic population structure. The estimated effective population size of only around three lions in the Sheep Range was particularly low.

In contrast, the lion population sampled along the eastern edge of the southern Sierra Nevada has large migratory deer populations as its primary prey base, but also bighorn sheep and introduced tule elk (*Cervus canadensis*) as large prey. In recent years, this ecosystem has supported resident lions and has been both a source and sink for migrants to neighboring areas that support mountain lions. Such metapopulation dynamics have helped to maximize genetic diversity in the Owens

Valley area (Davis et al. 2012). The number of lions that have resided in the region is close to the N_E estimate (Table 11), which speaks to the large amount of gene flow from neighboring regions, producing a maximally outbred population.

Male mountain lions frequently disperse long distances from their natal home ranges, as was demonstrated by the one young male lion that was caught in the Sheep Range and then traveled well outside of the study area. This behavior works to maximize outbreeding in an animal that often naturally exists at low population densities, and which otherwise would experience considerable inbreeding and consequent low heterozygosity. The lower heterozygosity and particularly low N_E estimate for the mountain lions sampled from the Sheep Range may reflect an isolated small population that does not receive enough immigration to maximize outbreeding. These results, however, might also reflect in part a small sample size that happened to include only closely related animals that represented only a portion of a larger population. A geographically wider sample would be useful to determine if this pattern is an artifact of the limited existing sample or reflects a larger pattern representing an isolated, small population.

BIGHORN SHEEP

Detection of Past Translocations

The large similarity in genetic population structure between the Spotted Range and River Mountains suggests that the translocation efforts in 1993 and 1996 were essentially a reintroduction of bighorn sheep to the Spotted Range as opposed to an augmentation. Microsatellite data were definitive in detecting little evidence of genes from native bighorn sheep in the current population within the range. The mtDNA data were concordant, but with the nuance that either a few native sheep were still present in the southern portion of the range at the time of translocations or that our sampling detected some rams that had since migrated into the range.

In contrast, we observed a lack of any genetic evidence of the 35 bighorn sheep translocated to the southern Sheep Range in 1998, which begs the question of why. The Arrow Canyon Range is adjacent to the Sheep Range, as described above. The 10 bighorn sheep translocated from the Arrow Canyon Range may have had little effect on genetic population structure in the Sheep Range because of potentially high natural gene flow between those ranges. Any potential genetic influence from those 10 sheep, based on microsatellites or mtDNA data, also was outside of our potential for detection given the lack of samples from the Arrow Canyon Range. Similarly, any influence of the 5 bighorn sheep released into the middle of the Sheep Range from the East Pahranagat Range in 1996 was likely washed out by the overall high levels of gene flow we documented across these ranges.

The same cannot be said for potential genes that came directly from the Muddy Mountains via the 10 bighorn sheep moved to the Sheep Range and indirectly from the Muddy and River Mountains through the 15 bighorn sheep moved from the Specter Range. Yet, our microsatellite assessments

did not detect any signal of these introductions in the genetic population structure of the Sheep Range. Our mtDNA data were inconclusive on this question because the limited resolution of this marker did not allow us to discern whether the high frequency of a particular haplotype of interest (haplotype M) was an artifact of translocations or a result of natural processes.

One possible explanation for the lack of a genetic signature from the translocations is that the population in the Sheep Range at the time was sufficiently high to greatly dilute the effect of such releases. Despite our large sample size, we simply may have not sampled any ancestors of those immigrants. Desert bighorn sheep ewes frequently live into their teens, even reaching mid-teens, and continue to reproduce at older ages. This means that some younger ewes released in the Sheep Range in 1998 could still have been alive when our sampling occurred for this study. Offspring of translocated bighorn sheep would have a considerably higher probability of being present when we sampled. Offspring derived from bighorn sheep moved from the Muddy Mountains would have a 50% signature of that ancestry in their microsatellite alleles, while their offspring would have a 25% signature. Such levels of genetic signature would be detectable by our STRUCTURE analysis, but were lacking in our results. Less certain is the level of contribution to the genetic structure of the Sheep Range of genes from the Muddy and River Mountains through bighorn sheep moved from the Specter Range. The generational reduction in genetic signature would likely have begun occurring in the Spector Range as Muddy and River Mountains bighorn sheep interbred with any native sheep in that region (dilution effect). We do not know, however, how many native bighorn sheep were present in the Spector Range when the augmentations occurred, and we have no data on the genetic structure of those sheep.

Other possible explanations for the lack of genetic evidence of translocations to the Sheep Range essentially suggest that these efforts were failures. One possibility was that the translocated sheep experienced a high mortality rate from exposure to microbes that cause respiratory disease that might have been present in the Sheep Range. The effect of respiratory disease is known to be potentially severe in bighorn sheep (Wehausen et al. 2011), and given previous declines in the Sheep Range, the presence of a local disease factor cannot simply be discounted. We know of no observations of a respiratory disease outbreak in the Sheep Range at that time, but recent disease testing of bighorn sheep in the range indicate high levels of exposure to respiratory microbes that appear to be causal agents or important predisposing factor for pneumonia (Longshore et al. *in prep.*).

Bighorn sheep released in a new landscape are also particularly susceptible to mountain lion predation because of a lack of knowledge of the habitat. Such predation susceptibility would be even stronger if the bighorn sheep came from ranges largely lacking that predator, such as the River and Muddy Mountains. This possibly has some support from observations at that time. Of the 35 sheep released into the southern Sheep Range, 12 were fitted with radio-collars and 2 of these were killed by mountain lions (see Longshore et al. *in prep*.). On the face of it, this level of predation does not seem to support the postulate that predation eliminated the bighorn sheep that were translocated. Mountain lions, however, likely killed a fair number of these sheep early-on, thus reducing the long-term genetic impact of the translocations on the population structure in the range.

Gene Flow

High levels of gene flow were clearly evident among the Sheep, Desert, and East Pahranagat ranges based on microsatellite data. The lower estimates for gene flow between those ranges and the Pintwater Range, however, were not expected given their close proximities. The F_{ST} values we measured between the Pintwater Range and the other ranges were similar to values documented further south in Nevada where there were significant barriers to gene flow (Jaeger and Wehausen 2012). One potential explanation is that the population genetic structure in the Pintwater Range has shifted because of gene flow from the Spotted Range where River Mountains sheep had been translocated. The potential influx of alleles from the River Mountains into the Pintwater Range would have the effect of increasing genetic distances with ranges further east. This explanation, however, was not supported by our STRUCTURE analysis which found little assignment of Pintwater samples to the cluster of samples from the River Mountains and Spotted Range. A more likely explanation for the distinction of the genetic population structure in the Pintwater Range may lie in gene flow involving bighorn sheep from mountainous areas to the immediate north that were not sampled in this study.

Genetic Diversity

The bighorn sheep population in the Sheep, Desert, and East Pahranagat ranges yielded the highest genetic diversity values that we have recorded for microsatellite data. This might reflect the influence of the augmentations in 1998; however, for that much influence to have occurred in just two generations, those translocated sheep would have to have constituted a large proportion of the population. If that had been the case, we would likely have detected that ancestry in our analyses, which we did not. An alternative explanation for the high genetic diversity in the Sheep, Desert, and East Pahranagat ranges is simply that the Sheep Range is a particularly large and high elevation desert mountain range that is well connected with neighboring ranges occupied by bighorn sheep. Previous research in the Mojave Desert of California found that gene flow in desert bighorn sheep was inversely related to distance between mountain ranges (Epps et al. 2005, 2007), and that genetic diversity in populations was related to the amount of connectivity with other populations and to the maximum elevation of mountain ranges (Epps et al. 2006). Populations in higher elevation ranges also have lower extinction probabilities, and thus lower extinction frequencies (Epps et al. 2004), which also preserves genetic diversity. Elevation is associated with higher levels of precipitation, lower temperatures, and more dependable water availability in the Mojave Desert (Epps et al. 2004). Higher precipitation and lower evapotranspiration results in increased diversity of vegetation assemblages and likely higher forage quality. The bighorn sheep population in the Sheep Range likely benefited from such conditions, allowing for long-term persistence and the resulting accumulation of genetic diversity.

In a larger temporal and management context, large, well connected, higher elevation systems maintain core bighorn sheep populations within metapopulations. Such core populations are more likely to persist through challenges caused by extreme climatic episodes and other variation, such as periods of disease or high predation. As such, these populations should be given special

management status. The protection of the Sheep Range and the associated ranges within a federal refuge is particularly appropriate.

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